Int J Clin Exp Med 2015;8(6):10204-10212
www.ijcem.com /ISSN:1940-5901/IJCEM0008463

Original Article

Evaluation of levator ani with no defect on elastography in women with POP

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Received March 26, 2015; Accepted June 4, 2015; Epub June 15, 2015; Published June 30, 2015

Abstract: Objective: It is difficult to evaluate the function of levator ani with no defect encountered in routine practice. Elastography is a new adjunct to real-time ultrasound imaging that overlays traditional B-mode imaging with a color graphic representation of tissue elasticity. The aim of this study is to specifically evaluate the function of levator ani with no defect by ES (elastography). Methods: This was a prospective, observational study. All patients were examined by MRI and the cases with levator ani defect were excluded. The included cases underwent conventional translabial ultrasound, and then ultrasound ES. A little levator ani were biopsied for immunohistochemistry check in the patients who underwent surgery. Results: Of the 52 cases with no levator ani defects, 22 had an elastography score of 1, 20 had a score of 2, 8 had a score of 3 and 2 had a score of 4 in levator ani. Cases of ES1 and ES2 were more than ES3 and ES4 (P = 0.004). The immunohistochemistry check indicated that 12 patients who underwent surgery showed weak expression and the other 4 patients showed moderate expression of collagen type I in levator ani, compared with 2 controls showing strong expressions. Conclusions: Elastography can assess the change of elasticity function of levator ani though the structure has no defect which was detected by MRI and conventional ultrasound. And probably the change of function of levator ani was earlier than structure abnormity in POP.

Keywords: Elastography, levator ani, ultrasound, pelvic organ prolapse

Introduction

Pelvic organ prolapse (POP) is a condition affecting more than half of the women above age 40 [1]. The pubovisceral or puborectalis/pubococcygeus muscle complex is thought to play a major role in pelvic floor dysfunction. It is also known that levator ani defects is associated with pelvic floor disorders, specifically POP and incontinence [2-4]. Recent work has described the appearance of the levator ani muscle on ultrasound (US) [5] and magnetic resonance imaging (MRI) [6], making it is possible to evaluate the morphology of the muscle specifically. However, to date the assessment of function of levator ani has not been reported.

Considering that echogenicity and the mechanical properties of body tissues are not correlated, a new dynamic tool to bridge the gap between ultrasound imaging and tissue structure and/or pathology, namely ultrasound elastography (ES), has been proposed and implemented. It is considered a novel technique that can be used to assess tissue elasticity by detecting tissue deformities occurring after sequential movements of compression and relaxation determined by the operator through the ultrasound probe. Ultrasound ES allows noninvasive evaluation of tissue elasticity using conventional real-time ultrasound equipment with modified software [7]. The different colors express different elasticity degrees, usually varying from red (highly elastic tissue) to blue (barely elastic tissue) while the other colors represent intermediate degrees of stiffness. In softer and highly elastic tissue, applied pressure causes the tissue to compress, with the tissue nearest the transducer compressing or moving the most and the tissue further away from the transducer moving less or not at all.

Table 1. Elastography scoring system for cervical lymph nodes (ES 1-4) and breast lesions (Score 1-5)

Elastographic score	Overall impression	Elastographic appearance
ES 1	soft	Predominant purple, green or yellow with less than 10% displaying red. The node is indistinguishable from surrounding tissues.
ES 2	moderately soft	Predominant yellow or green and with red areas comprising between 10% and 50%. The node is partially delineated from surrounding tissues.
ES 3	moderately soft	Predominant red and with yellow or green areas comprising between 10% and 50%. The node is partially delineated from surrounding tissues.
ES 4	stiff	Predominant red and with less than 10% appearing yellow or green. The node is distinguishable from surrounding tissues.

Figure 1. A patient with MRI showing a discontinuity (arrow) between the inferior pubic rami and the puborectalis muscle. The patient with levator ani defect was excluded.

A growing number of non-oncological applications for ultrasound ES are being explored including for differentiate malignant from benign lesions in the breast, prostate, lymph nodes, liver, gastrointestinal tract, cervix and gynecologic oncology [8-13]. However, to date, there have been no studies evaluating the utility of ultrasound ES for the function of levator ani with no defect encountered in routine practice, which was the purpose of the current study.

The aim of this prospective observational study is to specifically evaluated the function of levator ani with no defect by ES and compare it with MRI. We hypothesize that ES plays an important role in assessment of levator ani with no defect, especially in its function, and it was more effective than MRI.

Materials and methods

Patients

This was a prospective, observational study. The data were obtained at the Obstetrics and Gynecology Hospital of Fudan University, Shanghai, P. R. China from January 2011 to June 2013. The study design and protocol were approved by our Institutional Review Board, and all patients gave written informed consent after the nature of the procedure was explained fully.

Included patients were those who underwent a standard pelvic examination, POP quantification (POP-Q) examination, a MRI scan, translabial ultrasound and elastography (ES). Physical examination had been performed by 2 fellowship-trained urogynecologists. POP will be staged according to the POP-Q staging system of the International Continence Society [14]. Inclusion criteria for case subjects were twofold. First, subjects had to have no levator ani defects on MRI. Secondly, they had to have clinically pelvic floor dysfunction (POP-Q stage ≥ I) detected by urogynecologists. Women were excluded if they had previous surgery for pelvic floor dysfunction (e.g., pelvic organ prolapse, urinary incontinence, or fecal incontinence). Women who had undergone hysterectomy were eligible if the surgery had been at least 2 years prior to enrollment and if the indication for surgery was not pelvic floor dysfunction. Inclusion criteria for control subjects were (1) no levator ani defects detected by MRI (2) normal pelvic organ support on exam (POP-Q stage is 0). In this study, controls were two patients random selected who had vulval tumor.

MRI examination

All patients were examined by MRI in the T1-weighted images. Levator ani defect as

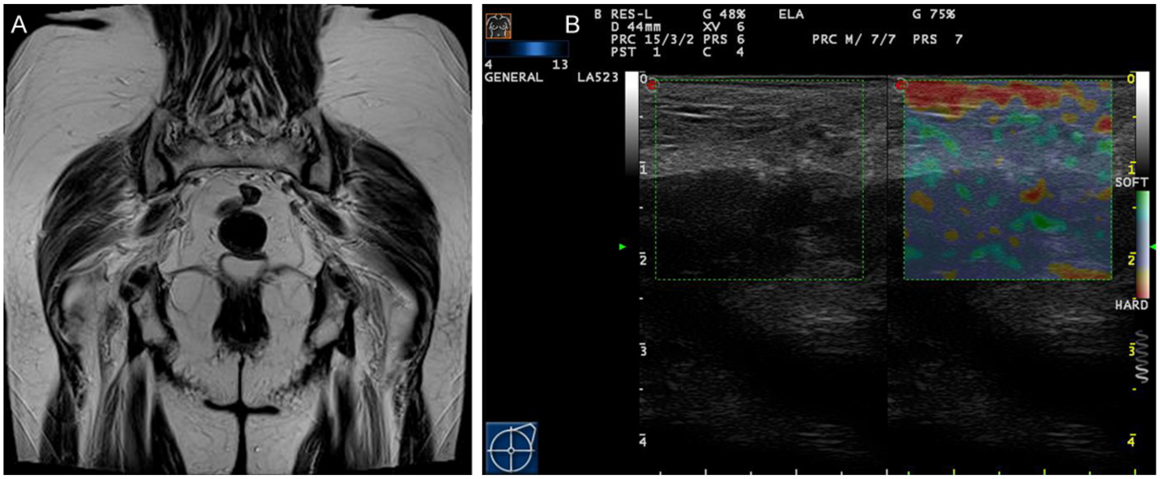

Figure 2. Case 1. A patient of levator ani with no defect detected by MRI (A). ES showed predominantly purple, green or yellow with less than 10% displaying red, scored as ES1 (B).

described by Dietz and Simpson [15] was defined as "a discontinuity between the inferior pubic rami and the puborectalis muscle". Determination of levator ani muscle defect status was performed by two examiners, blinded to subject demographic status. Each examiner independently graded levator ani defect status on MR images. The left and right levator ani muscles were scored separately. If levator ani defect was detected by MRI, the case was excluded.

ES examination

After MRI scan, all patients with no levator ani defect underwent conventional translabial ultrasound, and then ultrasound ES. All procedures were carried out by a radiologist with a longstanding experience (M.D., with 5 years experience in sonography and has been specialized in elastography for the last 1 year) of translabial ultrasound.

Imaging was performed with the patient supine and after bladder emptying. All assessments were conducted on maximal Valsalva. All patients underwent imaging on the system (Mylab 70, Esaote, Genoa, Italy) using translabial 10 MHz probe. First, using conventional translabial ultrasound, levator ani was located and assessed for the overall ultrasound appearance.

In each scanning plane selected for documentation with conventional ultrasound, translabial ES was also performed immediately after acquisition of the conventional grayscale ultrasound image. Then the real-time elastogram and the grayscale ultrasound image were displayed simultaneously. Using a freehand technique, the examiner manually applied slight, constant vertical compression along the major axis of the muscle.

With the use of the pressure indicator, optimal elastograms based on uniform tissue compressions were achieved. The information of the tissue elasticity was superimposed over the sonogram and displayed in color, with green indicating medium tissue stiffness, red indicating hard tissue and blue indicating soft tissue. Elasticity images were evaluated based on the conventional ultrasound presentation. All data collection and imaging analysis were performed prospectively by another radiologist (M.D., with 5 years experience in sonography and has been specialized in elastography for the last 1 year) who was blinded to the final diagnoses.

Due to lack of universally accepted criteria for scoring elastograms of levator ani in the published literature and our study was a new research, for easy to image analysis, elastograms were graded on a simplified 4-point scale (ES 1-4) (**Table 1**). This scale was adapted from previous studies of thyroid ultrasound ES [16].

Immunohistochemistry

If the patients underwent surgery, a little levator ani were biopsied for immunohistochemis-

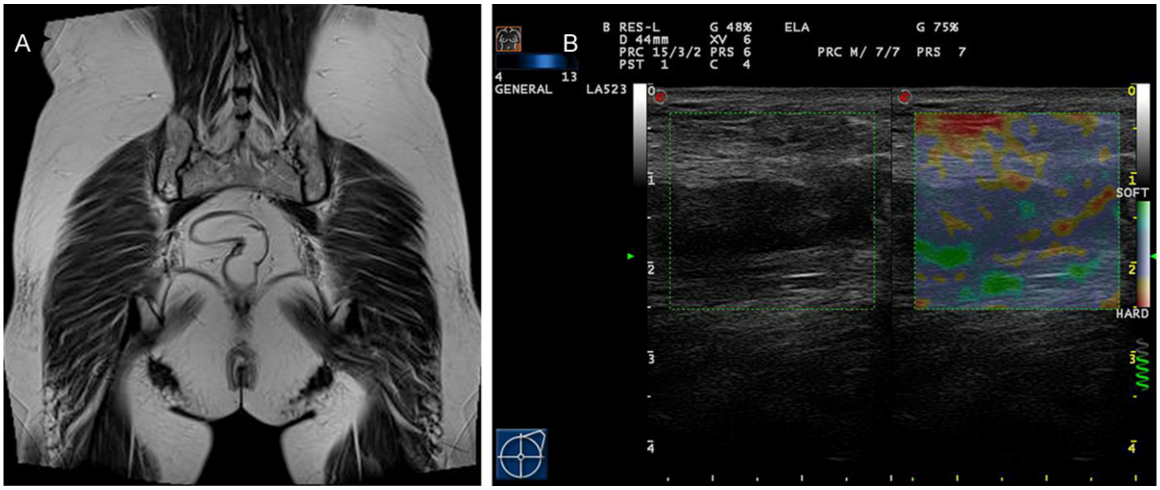

Figure 3. Case 2. A patient of levator ani with no defect detected by MRI (A). ES showed red areas comprising between 10% and 50%, scored as ES2 (B).

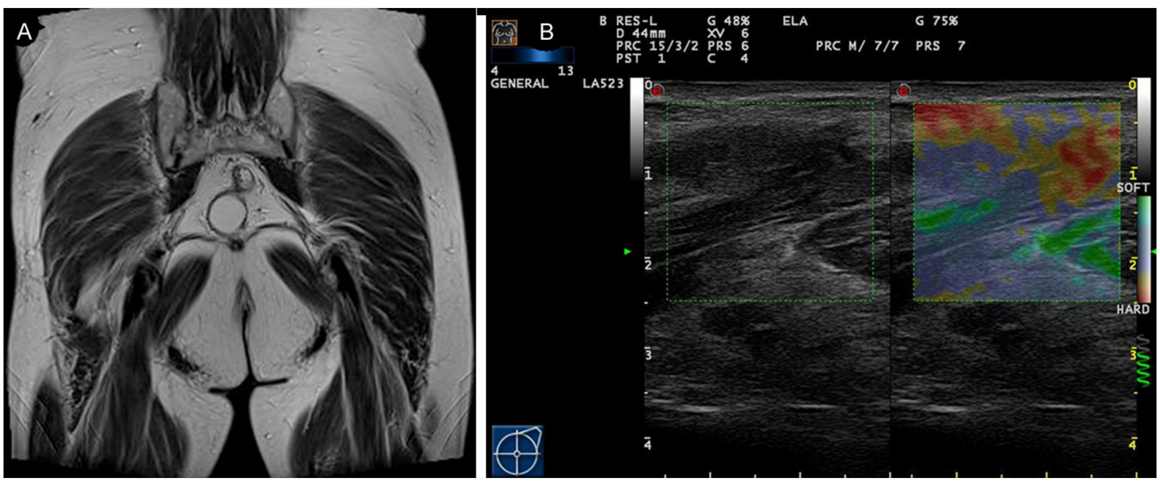

Figure 4. Case 3. A patient of levator ani with no defect detected by MRI (A). ES showed predominantly red and with yellow or green areas comprising between 10% and 50%, scored as ES3 (B).

try check. Formalin-fixed, paraffin-embedded specimens were sliced into 4-μm sections, placed on glass slides, allowed to dry at 37°Cfor 12 h. The primary antibodies used in this study was mouse monoclonal antibody against collagen type I (Abcam, Cambridge, UK; dilution 1:1500). Each sample was cooled, washed with phosphate-buffered saline (PBS) and distilled water, and incubated with H_2O_2 to block endogenous peroxidase for 15 min. Specimens were subsequently rinsed with PBS and incubated in a humid dark chamber at room temperature for 30 min with binding antibodies. Next, specimens were washed with PBS and incubated for 30 min with streptavidin. Specimens were then rinsed again with PBS and incubated with chromogen diaminobenzidine (DAKO, Glostrup, Denmark) in a heating steamer for 15 min. After this procedure, the slides were counterstained with hematoxylin, dehydrated in a graded alcohol series, cleared with xylol, and covered with balsam and glass. Two pathologists, blinded to the clinical diagnosis, analyzed each slide twice using a light microscope (Olympus, Tokyo, Japan). A score was applied to classify the intensity of staining: weak (1 +), moderate (2 +) or strong (3 +). This was based on interstitial staining for collagen type I [17]. Both positive and negative controls were performed. The positive control slides for collagen type I were prepared from skin. The negative control slides were prepared from the

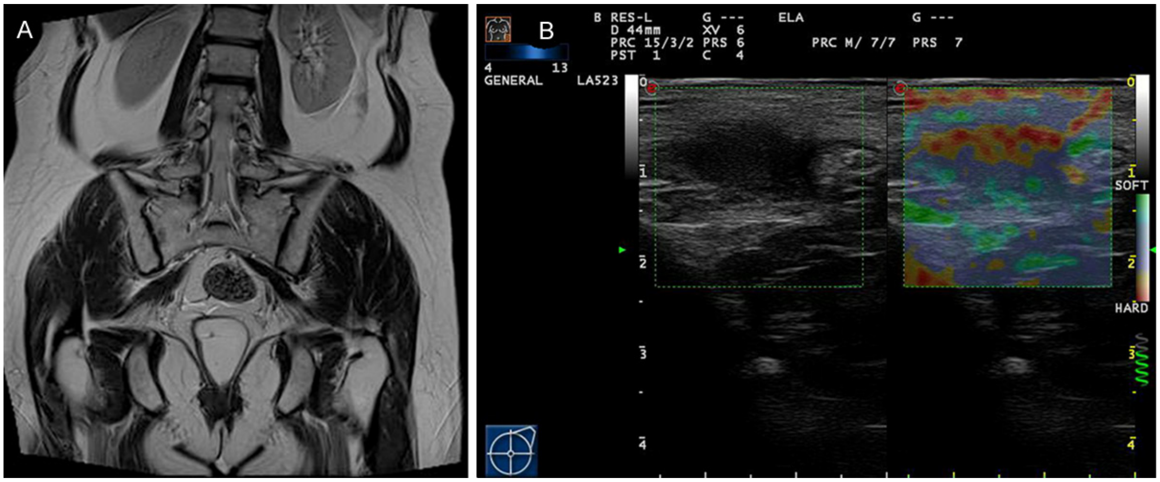

Figure 5. Case 4. A patient of levator ani with no defect detected by MRI (A). ES showed predominantly red and is clearly distinguishable, scored as ES4 (B).

Table 2. Elasticity scores in POP-Q stages (n = 52)

Elasticity score	stage I	stage II	stage III	stage IV
Score 1	9	7	2	4
Score 2	9	5	5	1
Score 3	4	3	1	0
Score 4	1	1	0	0
Total	23	16	8	5

same tissue block as the specimen, following the same procedure but with omission of the primary antibody.

Results

Of the 78 consecutive women who had clinically pelvic floor dysfunction detected by urogynecologists seen for assessment, 22 (28.2%) were excluded from further analysis because they had levator ani defect by MRI (**Figure 1**). A further 4 (5.1%) women were excluded because they were either not examined clinically due to vaginal scarring, atrophy or refusal (n = 3), or not imaged satisfactorily (n = 1). All analyses presented here refer to the remaining 52 (66.7%) women. Mean age was 58 years (range 45-83 years), and median parity was 2 (range 0-5). Prolapse with POP-Q stage I was diagnosed in 23 women (44.2%), 16 cases (30.8%) was stage II, 8 cases (15.4%) was stage III and 5 cases (9.6%) was stage IV.

Of the 52 cases with no levator ani defects, 22 had a elastography score of 1 (**Figure 2**), 20 had a score of 2 (**Figure 3**), 8 had a score of 3 (**Figure 4**) and 2 had a score of 4 (**Figure 5**) in levator ani evaluated by ES. Cases of ES1 and ES2 were more than ES3 and ES4 (P = 0.004). The distributions of elasticity scores for POP-Q stage I, II, III and IV are shown in **Table 2**. Of the 23 stage I cases, 9 (39.1%) had a score of 1, 9 (39.1%) had a score of 2, 4 (17.4%) had a score of 3 and 1 (4.3%) had a score of 4. Of the 16 stage II cases, 7 (43.8%) had a score of 1, 5 (31.3%) had a score of 2, 3 (18.8%) had a score of 3 and 1 (6.3%) had a score of 4. Of the 8 stage III cases, 2 (25.0%) had a score of 1, 5 (62.5%) had a score of 2, 1 (12.5%) had a score of 3 and none had a score of 4. Of the 5 stage IV cases, 4 (80.0%) had a score of 1, 1 (20.0%) had a score of 2, none had a score of 3 or 4. The 2 cases of controls had a score of 4 in levator ani (**Figure 6**).

1 case of stage I, 13 cases of stage II, 7 cases of stage III, all the cases of stage IV patients and 2 cases of controls underwent surgery. A little levator ani tissues were selected for immunohistochemical examination during the operation in 16 patients and 2 controls. 2 controls showed strong expression of collagen type I in levator ani (**Figure 7**). 12 patients showed weak expression and the other 4 showed moderate expression of collagen type I in levator ani (**Figure 8**).

Discussion

It has been established that levator ani muscle injury increases the risk of pelvic organ pro-

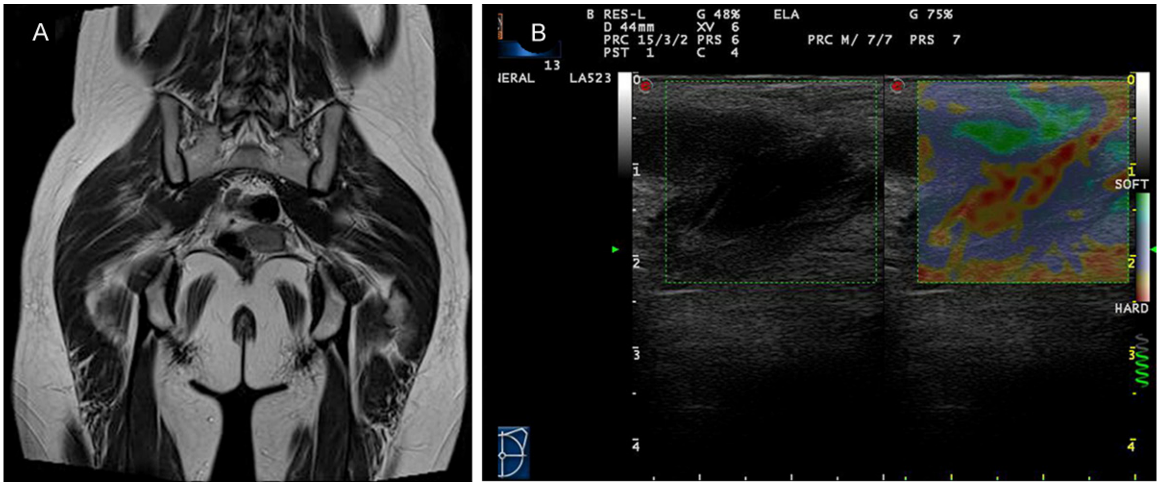

Figure 6. A case of levator ani with no defect detected by MRI (A). ES showed predominantly red and is clearly distinguishable, scored as ES4 (B). The case was control.

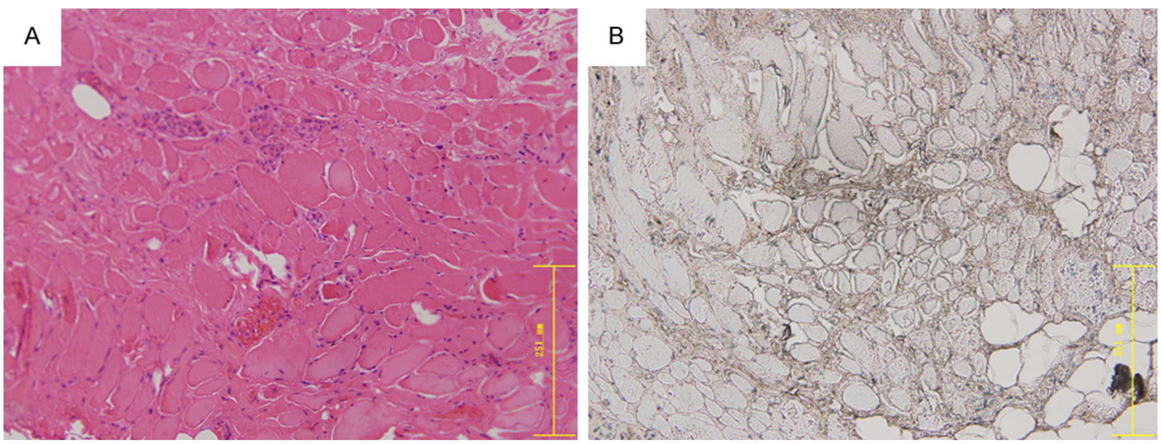

Figure 7. The control case with ES4. Hematoxylin & eosin staining (A), immunohistochemical examination of collagen type I (B) of levator ani. It had the abundant collagen tissues.

lapse (POP) [15]. Women with levator avulsion defects were about twice as likely to show pelvic organ prolapse of stage II or higher than those without. This effect is mainly due to an increased risk of cystocele and uterine prolapse. So amounts of researches of interdisciplinary imaging perspective on the pelvic floor focused on levator ani defects.

The absence of the levator ani muscle has been denoted as a defect or an avulsion attributed to many different causes such as labor and delivery, aging, or hormonal changes. Hudson referred to these changes collectively as "pelvic floor deficiency" [18]. To detect such birth-related injuries to the levator ani muscles, modern imaging techniques such as MRI [19], transperineal ultrasonography [20], and endovaginal ultrasonography [21] have been used.

Recent advancements in imaging allow assessment of the levator ani muscle imaging with 3D pelvic floor ultrasound, comparing it to MR imaging as the reference standard. Because accurate assessment of pelvic floor injury may help explain symptoms and potentially guide future treatment options, it is important to study on imaging in detecting these levator defects. Defects of the puborectalis muscle can be diagnosed with 2D US [22]. The finding of a discontinuity between the hyperechogenic muscle and the pelvic sidewall is moderately reproducible and agrees moderately well with palpation and 3D US. Moreover, using 3D pelvic

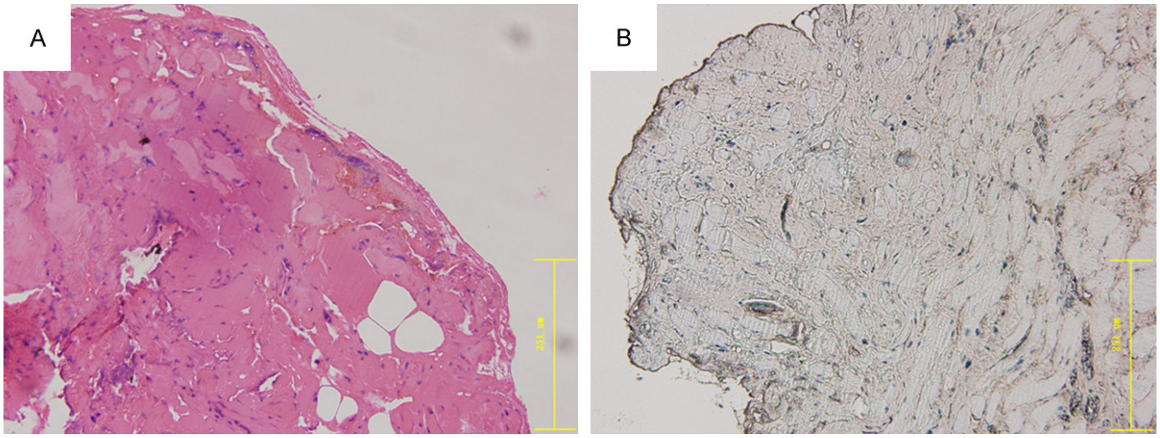

Figure 8. A patient (Case 1) with ES1. hematoxylin-eosin staining (A), immunohistochemical examination of collagen type I (B) of levator ani. It had the deficient collagen tissue.

ultrasound for diagnosing levator defects is non-invasive, patient friendly, less expensive and it has practical advantages like shorter examination time. In addition, depth and width of levator ani defects can be quantified by tomographic pelvic floor ultrasound [23]. These parameters are associated with the likelihood of symptoms of prolapse and cystocele descent.

However, a PubMed search showed no earlier studies dealing with the evaluation of levator ani elasticity by ES and the estimation of levator ani dysfunction, especially levator ani with no defects. We suggested that ultrasound examination should not only evaluate whether or not the levator ani defects but also estimate the elasticity of the muscle. In our study, of the 52 cases with no levator ani defects, cases of ES1 and ES2 were more than ES3 and ES4. It means that elastography can assess the change of elasticity function of levator ani though the structure has not defect which was detected by MRI and conventional ultrasound. And probably the change of function was earlier than structure abnormity in POP. In fact, slight POPs are relatively easy to interfere. So the imaging evaluation is important and necessary. Our study suggests that low elasticity of levator ani with no defects should be considered to be risk factors for the development of POP. And the clinical implication of the present study could be to interfere as soon as possible, such as pelvic floor muscle strength training.

In our opinion, levator ani defect is not the only index of pelvic floor dysfunction. Levator ani, especially with no defect but have abnormal elasticity detected by ES should be considered to have further follow-up investigation and whether women with levator ani abnormality are more likely to develop a severe prolapse.

The purpose of assessment of levator ani by MRI in our study was that it was a good tool for evaluating levator ani defect. Women with prolapse were more likely to have major levator ani defects than controls detected by MRI [3]. Additionally, the muscle does not appear to be thinner or more atrophic in individuals with defects and prolapse compared with women with normal muscles and support [24].

Translabial ultrasound, especially 4D real-time imaging, has the major advantage of providing a global view of the entire pelvic floor, from the symphysis to the anorectum, and includes the lower aspects of the levator ani muscle. As a result, the modality is optimally suited for interdisciplinary assessment and communication. Since that, in our study, translabial ES was chosen [25].

Conventional ultrasound and MRI cannot reveal the imaging of the elasticity function, this may lead to treat late, and subsequently disease develops. Immunohistochemical evaluation confirmed it. The preliminary findings are encouraging, as they suggest that ES technique may be useful as an adjunct to conventional or 3D ultrasound in the assessment of levator ani elasticity function. Thus, clinical intervention can be planned as early as possible, particularly in the women who are fear of surgery.

Of course the aim of this study is not to compare ES against conventional ultrasound and MRI but to evaluate any potential role as an adjunct. There are several limitations that should be kept in mind in evaluating the results of this study. Given our small sample size, the results are not representative of what would occur in the population, and further research would be needed in order to address that issue. The assessment used was subjective, based on the authors' extensive experience with examining the levator ani, and specific measurements of levator ani muscle thickness, width and length were not made. This was done because of the highly variable morphology of the muscle, thus making it very difficult to quantify muscle bulk. Whether the bulk was consistent with elasticity was not known. Also, it is not possible for the investigators to be completely blinded to subject status, because all the patients were POPs and some of them could be found uterine prolapse when they were evaluated. Finally, future studies involving the use of ES both before and after surgery are needed to investigate the effect of surgery in a prospective fashion.

In conclusion, our result showed that ES can evaluate the function and elasticity of levator ani with no defect. More number of sample about levator ani with no defect in POPs with assessment by elastography is needed to perform to certify its advantages.

Disclosure of conflict of interest

None.

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